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Evaluation of the effects of anti-psychotic drugs on the expression of CD68 on the peripheral blood monocytes of Alzheimer patients with psychotic symptoms

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ABSTRACT

Introduction: Previous studies approved the important roles of CD68, as scavenger receptors, in Alzheimer's disease (AD). The aim of this study was to evaluate the effect of treatment with anti-psychotic drugs and vitamin B12 on the expression levels of CD68 in monocytes of psychotic AD patients.
Material and methods: Expression of CD68 on the monocytes was evaluated in the following groups: 1. age and sex matched healthy controls (Group 1), 2. non-psychotic AD patients (Group 2), 3. psychotic AD patients (Group 3), 4. psychotic AD patients treated with Risperidone (Group 4), 5. psychotic AD patients treated with Risperidone plus vitamin B12 (Group 5), 6. psychotic AD patients treated with Quetiapine (Group 6), psychotic AD patients treated with Quetiapine plus vitamin B12 (Group 7). The expression of CD68 has been performed using flow cytometry technique.
Results: The results showed that CD68 levels were significantly increased in AD patients in comparison to healthy controls and in psychotic AD patients in comparison to non-psychotic AD patients. Treatment with anti-psychotic drugs decreased the expression of CD68. Expression of CD68 has a positive correlation with pain, dementia and mental disorders symptoms in psychotic AD patients.
Discussion: CD68 may play key roles in the pathogenesis of AD and its complications may be via induction of inflammation. Therefore, it may be concluded that CD68 may be considered as a risk factor for development of AD and also psychotic symptoms in the patients.

1. Introduction

Alzheimer's disease (AD) is a type of dementia, associated with a significant reduction in the individual ability and progressive deterioration of cognitive function and expression [1]. According to a study conducted at Johns Hopkins University, the prevalence rate of AD will be increased to 1 in 85 living people by the year 2050 [2,3]. Emerging evidence suggests that inflammation has a critical role in the disease pathogenesis and hence, understanding and control of interactions between the immune system and the nervous system may be essential to prevent and delay most late-onset central nervous system (CNS) diseases [4]. The microglia are the most important immune system cells in the brain, derived from the monocytes and play significant roles in the Alzheimer pathogenesis [5]. Therefore, it appears that the functions of peripheral blood monocytes can be associated with alteration in the microglia functions [5]. Monocytes play dual roles in the immune responses from inflammatory to anti-inflammatory functions [6,7].

Based on the fact that AD patients suffer from a chronic inflammation, it appears that the inflammatory functions of monocytes may be considered as a risk factor for deterioration of AD pathogenesis [7,8].

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Table 1
Comparison of gender and age distribution among evaluated groups.

Groups		1	2	3	4	5	6	7	p-Value
Variable		(n = 19)	(n = 15)	(n = 21)	(n = 10)	(n = 10)	(n = 16)	(n = 11)	
Gender	Male	8 (42.1)	6 (40)	9 (42.8)	6 (60)	6 (60)	6 (37.5)	5 (45.4)	0.703
	Female	11 (57.9)	9 (60)	12 (57.2)	4 (40)	4 (40)	10 (62.5)	6 (54.6)	
Age		75.27 ± 6.18	74.64 ± 6.04	78.89 ± 6.69	75.78 ± 9.48	75.50 ± 5.71	79.93 ± 3.00	79.50 ± 2.84	0.120

Fisher's exact test revealed that the groups did not differ regarding gender and age distribution.
Data are presented as mean ± SD or n (%).
Group 1: healthy controls, group 2: AD patients without psychotic symptoms, group 3: AD patients with psychotic symptoms, group 4: AD patients with psychotic symptoms treated with Risperidone, group 5: AD patients with psychotic symptoms treated with Risperidone and vitamin B12, group 6: AD patients with psychotic symptoms treated with Quetiapine, group 7: AD patients with psychotic symptoms treated with Quetiapine and vitamin B12.
VAS: Visual Analogue Scale, CGI: Clinical Global Impression, BPRS: Brief Psychiatric Rating Scale, MMSE: Mini–Mental State Examination.

Monocytes elicit their inflammatory functions via several molecules including scavenger receptors (SRs) [9]. SRs were initially identified on the basis of their biochemical ability to recognize and bind different modified forms of LDL [9,10]. Accordingly, chronic inflammation can lead to some complications in AD patients [11], and based on the pro-inflammatory functions of CD68, it may be hypothesized that the molecule may participate in the pathogenesis of AD. Thus, one aim of this study was to evaluate the glycoprotein levels of CD68 on the monocytes of AD patients in comparison to healthy controls. Additionally, it has been reported that AD patients are divided into two main groups including psychotic and non-psychotic [12,13].

The psychotic patients were treated with anti-psychotic drugs such as Risperidone and Quetiapine [14,15]. It appears that the drugs may alter expression of pro-inflammatory molecules including cell surface receptors. Thus, it has been hypothesized that the drugs may alter expression of CD68, as an inflammatory molecule, on the monocytes [16,17]. Therefore, another aim of this project was to compare the effects of treatment with Risperidone and Quetiapine on the expression of CD68 in psychotic patients and also compare the expression of the molecules between untreated psychotic and non-psychotic patients. Recent studies have shown that vitamin B12 plays an essential role in reducing brain and nervous system disorders [18]. However, the exact mechanism of action of these drugs is unknown. According to the description above, another purpose of this study was to evaluate CD68 expression on the surface of peripheral blood monocytes in AD patients with psychotic symptoms (Creavin et al.) receiving Risperidone, Quetiapine in associated with Vitamin B12.

Additionally, it has been demonstrated that Mini–Mental State Examination (MMSE, which is used for detection of dementia), Clinical Global Impression (CGI, which is used for detection of severity of mental disorders symptoms, treatment response and efficacy), Brief Psychiatric Rating Scale (BPRS, which is used for determination of psychiatric symptoms) and Visual Analogue Scale (VAS, which is used for detection of pain levels that are the questionnaires filled out by the AD patients with psychotic symptoms to evaluate the variables in the patients before and after treatment with anti-psychotic drugs) [19–22]. Thus, it has been hypothesized that the variables may be associated with expression of immune related molecules such as macrophages surface molecules. Based on the fact that CD68 is an important molecule that participates in the macrophages functions, alteration in the MMSE, CGI, BPRS and VAS variables may be associated with expression and functions of CD68. Therefore, a further aim of this study was to determine the relation between MMSE, CGI, BPRS and VAS with expression levels of CD68 in AD patients with psychotic symptoms.

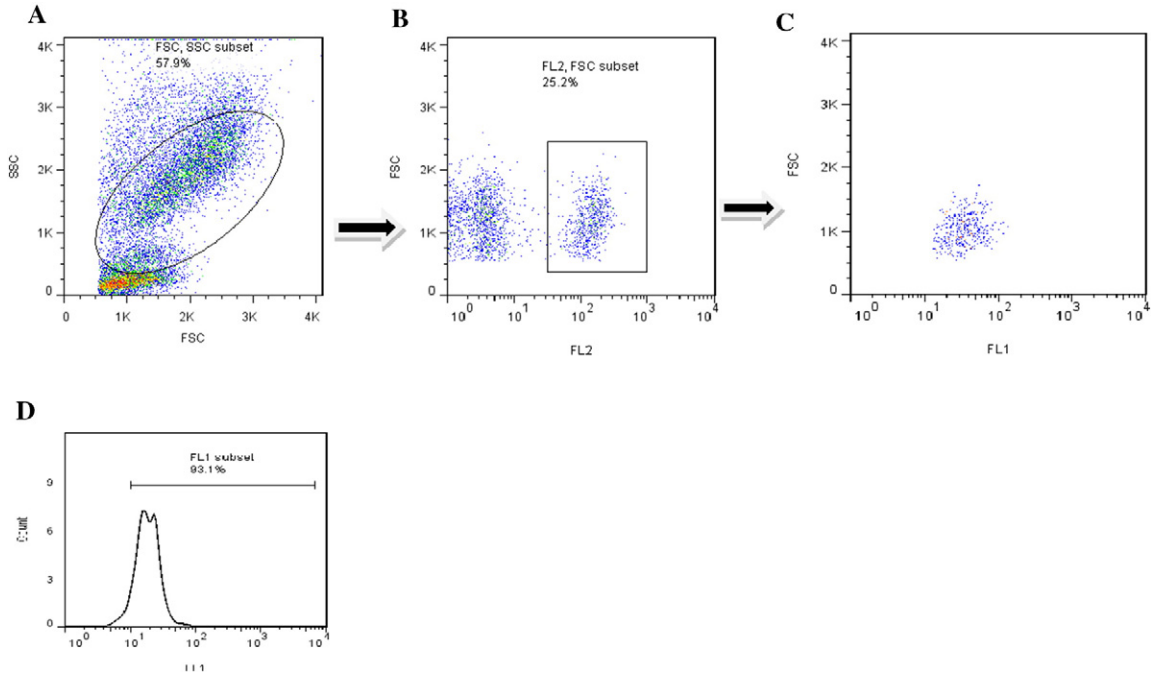

Fig. 1. The dot plots and histogram of $CD68^+$ monocytes from a participant. Panel A illustrates the dot plots using side scatter (SSC) and forward scatter (FSC) which has gaited the monocytes population. Panel B illustrates the dot plots using FSC and FL2 (CD14 positive cells which have been stained with PE color) which has been performed on the first gaited region. Panel C is created from CD14 positive cells which are drawn using FSC and FL2 (CD68 positive cells which have been stained with FITC color). Panel D, the Panel D illustrates that 93.1% of $CD14^+$ cells express CD68.

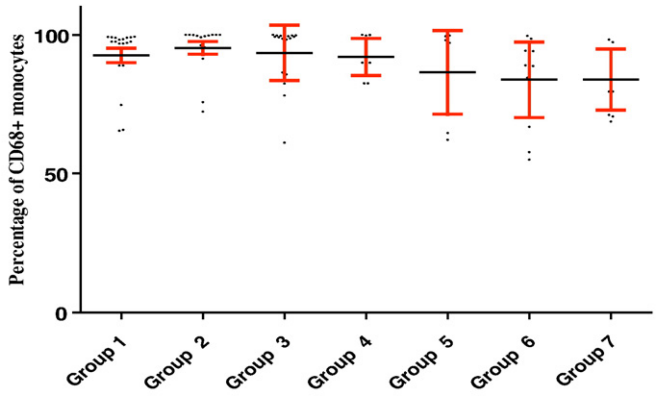

Fig. 2. Comparison of percentage of CD68 positive monocytes in the Alzheimer's patients and healthy controls and the effects of treatment with anti-psychotic drugs and vitamin B12 on the cell population. The results demonstrated that percentage of CD68 positive monocytes was significantly different among the participants ($p = 0.017$). Treatment of psychotic AD patients leads to decrease in percentage of CD68 positive monocytes. Although vitamin B12 could reduce the percentage of CD68 positive monocytes in group 5 when compared to group 4, the difference was not significant. Data table for "Mean ± SE" has been reported. Group 1: healthy controls, group 2: AD patients without psychotic symptoms, group 3: AD patients with psychotic symptoms, group 4: AD patients with psychotic symptoms treated with Risperidone, group 5: AD patients with psychotic symptoms treated with Risperidone and vitamin B12, group 6: AD patients with psychotic symptoms treated with Quetiapine, group 7: AD patients with psychotic symptoms treated with Quetiapine and vitamin B12.

2. Material and methods

2.1. Subjects

In this study, after collection of demographic information, 2 ml peripheral blood samples were collected in EDTA pre-treated tubes. The peripheral blood monocytes were stained with appropriate fluorescence conjugated antibodies (see next subsection) after treatment with a commercial red blood cell lysis buffer (eBiosciences, Spain). Accordingly, the protein levels of CD68 were evaluated on the peripheral blood monocytes in healthy control subjects (Group 1), AD patients without psychotic symptoms (Group 2), AD patients with psychotic symptoms (Group 3), AD patients with psychotic symptoms taking Risperidone (Group 4), AD patients with psychotic symptoms taking Risperidone and vitamin B12 (Group 5), AD patients with psychotic symptoms taking Quetiapine (Group 6) and AD patients with psychotic symptoms taking Quetiapine and vitamin B12 (Group 7). The including criteria were as follows: elderly people over 65 years old who have been referred to neurologists in Rafsanjan city and recognized as new cases of AD. An expert neurologist confirmed the psychotic symptoms according to the Guide of Prevention and Treatment in psychotic symptoms criteria [23,24]. The following exclusion criteria were used for selection of the patients: Severe pro-inflammatory diseases including infectious, cardiovascular, kidney or liver disorders, insulin-dependent diabetes, thyroid disorders untreated. The patients under administration of anti-inflammatory/immunosuppressant drugs (except acetylsalicylic acid) were also excluded from the investigation.

Peripheral blood was obtained from participants in pre-treated anticoagulant tubes for flow cytometry analysis.

MMSE, CGI, BPRS and VAS scales have been evaluated using the standard questionnaires [21,25–27].

The Ethical Board of the Shahid Sadoughi University of Medical Sciences has approved the protocol of this investigation by the following code: IR.SSU.MEDICINE.REC.1395.112. The ethical committee of Iranian Health Ministration also approved the protocol of the study with code: IRCT2016092125067N3. The participants have filled out the written informed consent before introduction to the project. Participants were selected with same gender and age to exclude the interfered factors (Table 1).

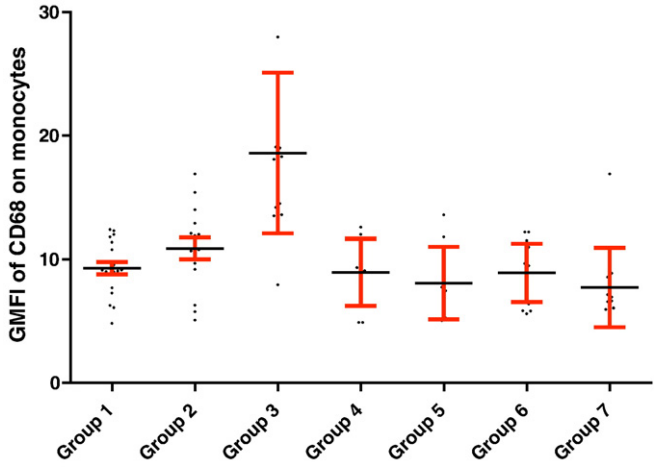

Fig. 3. Comparison of geometric mean fluorescence intensity (GMFI) levels of CD68 positive monocytes in the Alzheimer patients and healthy controls and the effects of treatment with anti-psychotic drugs and vitamin B12 on the CD68 expression. The results demonstrated that GMFI levels of CD68 positive monocytes were significantly different among the participants ($p < 0.001$). The GMFI of CD68 was decreased in the anti-psychotic treated patients when compared to non-treated psychotic patients. Although vitamin B12 decreased GMFI of CD68 on monocytes, the differences were not significant. Data table for "Mean ± SE" has been reported. Group 1: healthy controls, group 2: AD patients without psychotic symptoms, group 3: AD patients with psychotic symptoms, group 4: AD patients with psychotic symptoms treated with Risperidone, group 5: AD patients with psychotic symptoms treated with Risperidone and vitamin B12, group 6: AD patients with psychotic symptoms treated with Quetiapine, group 7: AD patients with psychotic symptoms treated with Quetiapine and vitamin B12.

2.2. Flow cytometry analysis

Expression of 68 molecules on the surface of peripheral blood monocytes were measured by direct staining of the cells after treatment with red blood cell lysis buffer with Phycoerythrin (PE) conjugated monoclonal antibodies against CD14 (clone: 61D3, isotype: mouse IgG1), fluorescein isothiocyanate (FITC) conjugated 68 (clone: eBioY1/82A (Y1/82A)) along with corresponded isotype-matched negative control (IgG1, clone: P3.6.2.8.1; IgM, IgG2b, κ, clone; eBMG2b). Accordingly, 100 μl of blood was added to flow cytometry tubes then 10 μl antibodies against CD14 labeled with PE was added to determine monocytes, next 10 μl antibodies against CD68 labeled with FITC was added to determine the percentage and intensity of CD68 expression and incubated in a dark place for 30 min. After that, 1 ml RBC lysis buffer was added to the tubes. A

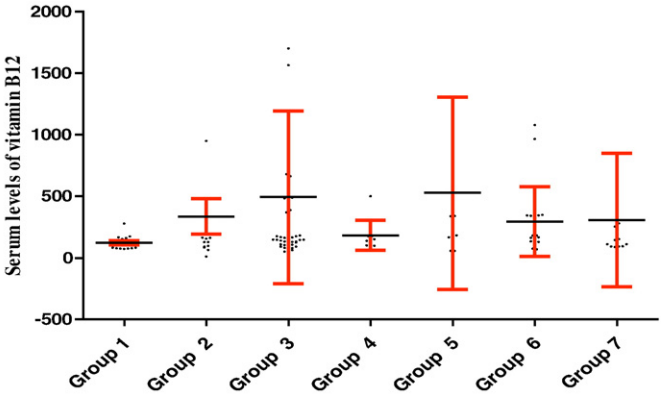

Fig. 4. Serum levels of vitamin B12 in the evaluated participants. The results demonstrated that serum levels of vitamin B12 were not different between groups. Group 1: healthy controls, group 2: AD patients without psychotic symptoms, group 3: AD patients with psychotic symptoms, group 4: AD patients with psychotic symptoms treated with Risperidone, group 5: AD patients with psychotic symptoms treated with Risperidone and vitamin B12, group 6: AD patients with psychotic symptoms treated with Quetiapine, group 7: AD patients with psychotic symptoms treated with Quetiapine and vitamin B12.

Table 2
Correlations among percentage of CD68 positive monocytes, GMFI of CD68 on monocytes and serum levels of vitamin B12 with age, VAS, CGI, BPRS and MMSE in group 3.

		Percentage of CD68 positive monocytes	CD68 gMIF	Vitamin B12 serum levels	Age	VAS	CGI	BPRS	MMSE
Percentage of CD68 positive monocytes	Spearman correlation	1	−0.567	0.104	−0.020	−0.126	0.024	−0.031	0.107
	p value		0.007	0.663	0.930	0.585	0.919	0.894	0.643
CD68 GMFI	Spearman correlation	−0.567	1	−0.035	−0.314	0.239	0.243	−0.064	−0.063
	p value	0.007		0.887	0.166	0.297	0.289	0.782	0.785
Vitamin B12 serum levels	Spearman correlation	0.104	−0.035	1	0.029	0.101	−0.285	−0.145	0.210
	p value	0.663	0.887		0.894	0.682	0.237	0.555	0.389

Spearman's correlation test revealed that the percentage of CD68 positive monocytes has a moderate positive correlation with concentration of CD68 ($r = -0.567$, $p = -0.007$).
GMFI: geometric mean fluorescence intensity, VAS: Visual Analogue Scale, CGI: Clinical Global Impression, BPRS: Brief Psychiatric Rating Scale, MMSE: Mini–Mental State Examination.

negative control using isotype control antibody was prepared to set up the protocol. Partec particle and cell sorting instrument (Münster, Germany) were used for analysis of the stained monocytes. In order to analyze the raw data, Flow Jo software has been used (Fig. 1). The antibodies were purchased from eBioscience Company (Spain) and staining was performed according to the manufacturer's instructions.

2.3. Statistical analysis

Data was analyzed using SPSS software version 16 (v16; SPSS Inc., Chicago, IL, USA). The differences of numeric variables across groups were evaluated using one-way ANOVA followed by Tukey's multiple comparisons test. Frequencies were compared using Fisher's exact test. The association between numeric variables was assessed using Spearman's correlation coefficient. Significance level was set at 0.05.

3. Results

The results demonstrated that the percentage of CD68 on monocytes of group 1, 2, 3, 4, 5, 6 and 7 was 92.53 ± 2.56, 95.25 ± 2.32, 93.47 ± 2.18, 91.79 ± 2.16, 86.01 ± 4.51, 83.87 ± 3.40, 83.78 ± 3.32, respectively (Fig. 2). Data are shown as mean ± SE. Statistical analysis revealed that the differences between groups regarding the percentage of CD68 on monocytes was significant ($p = 0.017$). Tukey's multiple comparisons test showed that the percentage of CD68 was decreased in the group 5 in comparison to group 4 and in group 6 and 7 when compared to non-treated AD patients and group 4 (Fig. 2).

The results demonstrated that the concentrations GMFI of CD68 on monocytes of groups was 9.29 ± 2.18, 10.90 ± 3.37, 18.98 ± 6.07, 9.01 ± 2.50, 7.99 ± 2.78, 8.93 ± 2.36, 7.74 ± 3.22, respectively. Analysis of GMFI of CD68 on monocytes showed that GMFI means were significantly increased in the group 3 in comparison to all groups ($p < 0.001$, Fig. 3). Treatment with Risperidone and Quetiapine decreased GMFI of CD68 in the AD psychotic patients. Vitamin B12 also decreased the GMFI of CD68 but it was not statistically significant.

The results revealed that serum levels of vitamin B12 do not differ among groups ($p = 0.298$, Fig. 4).

Statistical analysis revealed that there were not significant correlations between serum levels of vitamin B12 and percentage of CD68 positive monocytes ($r = -0.246$, $p = 0.418$). Also, there were no significant correlations between vitamin B12 and concentration of CD68 on monocytes ($r = -0.360$, $p = -0.226$) in group 1 and between serum levels of vitamin B12 and percentage of CD68 positive monocytes ($r = -0.242$, $p = 0.449$) and between vitamin B12 and concentration of CD68 on monocytes ($r = -0.252$, $p = 0.429$) in group 2.

Evaluation of correlation between variables in group 3 using Spearman's correlation test revealed that the percentage of CD68 positive monocytes has a moderate negative correlation with concentration of CD68 ($r = -0.567$, $p = -0.007$, Table 2).

Evaluation of group 4 regarding the correlation between percentage of CD68 positive monocytes and concentration of CD68 revealed that there is a perfect positive correlation between these variables ($r = 0.90$, $p < 0.001$) and a perfect negative relation between serum levels of vitamin B12 and CGI ($r = -0.825$, $p = -0.012$, Table 3).

Analysis of group 5 also showed that there is a good positive correlation between percentage of CD68 positive monocytes and concentration of CD68 ($r = 0.727$, $p = 0.017$), a moderate positive correlation between percentage of CD68 positive monocytes and age ($r = 0.518$, $p = 0.125$) and a poor correlation between percentage of CD68 positive monocytes and VAS ($r = 0.051$, $p = 0.629$, Table 4).

Statistical analysis revealed poor correlations between percentage of CD68 positive monocytes and intensity of CD68 with age, VAS, CGI, BPRS and MMSE in group 6 (Table 5).

Analysis of group 7 demonstrated there was a moderate to good positive correlation between percentage of CD68 positive monocytes and MMSE ($r = 0.621$, $p = 0.042$) and also between concentration of CD68 and CGI ($r = 0.560$, $p = 0.073$, Table 6). The results also demonstrated that there was a moderate negative correlation between serum levels of vitamin B12 and BPRS ($r = -0.560$, $p = 0.037$).

4. Discussion

Monocytes play critical roles in the pathology of brain inflammatory disorders [28]. The pathology of AD is due to an increase in amyloid B deposition more than monocyte phagocytosis [29]. CD68 as a scavenger receptor on monocytes may play a dual role in clearance of amyloid beta and induction of chronic inflammation.

Table 3
Correlations among percentage of CD68 positive monocytes, GMFI of CD68 and serum levels of vitamin B12 with age, VAS, CGI, BPRS and MMSE in group 4.

		Percentage of CD68 positive monocytes	CD68 GMIF	Vitamin B12 serum levels	Age	VAS	CGI	BPRS	MMSE
Percentage of CD68 positive monocytes	Spearman correlation	1	0.900	0.024	0.220	0.351	−0.027	0.092	0.038
	p value		0.000	0.955	0.540	0.320	0.941	0.801	0.918
CD68 GMFI	Spearman correlation	0.900	1	0.238	0.287	0.224	0.049	0.110	−0.052
	p value	0.000		0.570	0.422	0.534	0.892	0.762	0.886
Vitamin B12 serum levels	Spearman correlation	0.024	0.238	1	−0.386	0.135	−0.825	−0.108	0.626
	p value	0.955	0.570		0.346	0.750	0.012	0.799	0.097

Spearman's correlation test showed that there is a perfect positive relation between percentage of CD68 positive monocytes and GMFI of CD68 ($r = 0.90$, $p < 0.001$) and a perfect negative relation between serum levels of vitamin B12 and CGI ($r = -0.825$, $p = -0.012$).
GMFI: geometric mean fluorescence intensity, VAS: Visual Analogue Scale, CGI: Clinical Global Impression, BPRS: Brief Psychiatric Rating Scale, MMSE: Mini–Mental State Examination.

Table 4
Correlations among percentage of CD68 positive monocytes, GMFI of CD68 and serum levels of vitamin B12 with age, VAS, CGI, BPRS and MMSE group 5.

		Percentage of CD68 positive monocytes	CD68 GMIF	Vitamin B12 serum levels	Age	VAS	CGI	BPRS	MMSE
Percentage of CD68 positive monocytes	Spearman correlation	1	0.727	0.200	0.518	0.629	−0.041	0.036	0.150
	p value		0.017	0.606	0.125	0.051	0.911	0.922	0.680
CD68 GMFI	Spearman correlation	0.727	1	−0.050	0.422	0.252	0.089	−0.207	−0.030
	p value	0.017		0.898	0.225	0.483	0.807	0.567	0.935
Vitamin B12 serum levels	Spearman correlation	0.200	−0.050	1	0.151	0.632	−0.416	0.367	0.469
	p value	0.606	0.898		0.699	0.068	0.266	0.332	0.203

Spearman's correlation test showed that there is a good positive correlation between percentage of CD68 positive monocytes and GMFI of CD68 ($r = 0.727$, $p = 0.017$), a moderate positive correlation between percentage of CD68 positive monocytes and age ($r = 0.518$, $p = 0.125$) and a poor positive correlation between percentage of CD68 positive monocytes and VAS ($r = 0.629$, $p = 0.051$).
GMFI: geometric mean fluorescence intensity, VAS: Visual Analogue Scale, CGI: Clinical Global Impression, BPRS: Brief Psychiatric Rating Scale, MMSE: Mini–Mental State Examination.

Data analysis showed that the percentage of CD68 positive monocytes was significantly decreased after treatment of psychotic patients with anti-psychotic drugs (Risperidone and Quetiapine). Interestingly, although it was not significant, vitamin B12 can reduce the percentage of CD68 positive monocytes. Additionally, the results showed that GMFI of CD68 was significantly increased in AD patients in comparison to healthy controls and also in psychotic patients when compared to AD non-psychotic patients. Moreover, treatment with Risperidone and Quetiapine reduced GMFI of CD68. Vitamin B12 also decreased the expression of CD68 in patients treated with anti-psychotic drugs. Therefore, it appears that although the percentage of CD68 positive monocytes did not differ between healthy controls and AD patients, the concentration of CD68 was significantly increased in AD patients. Thus, it may be concluded that CD68 may participate in the pathogenesis of AD. Additionally, the intensity of CD68 was significantly increased in psychotic patients when compared to AD non-psychotic patients. Hence, it could be hypothesized that the molecule may be an important factor for induction of psychotic symptoms in AD patients. On the other hand, previous investigations revealed that treatments with anti-psychotic drugs (Risperidone and Quetiapine) are associated with improvement of psychotic symptoms [30–32] which is in concordance with decreased CD68 concentrations and percentage of CD68 positive monocytes in the current study. Thus, it may be concluded that CD68 can participate in induction of psychotic symptoms.

To the best of our knowledge, the current investigation is the first study which evaluated the percentage of CD68 positive peripheral blood monocytes and concentration of CD68 on the monocytes in AD patients. However, Argiles et al., reported that expression of CD68 has a positive relation with haemodialysis-associated amyloidosis [33], which confirms the roles of CD68 in response to β-amyloids that plays key roles in the pathogenesis of AD [34,35]. Additionally, previous investigations have approved the roles of CD68 positive microglia cells in the pathogenesis of AD [36–38]. Our results confirmed the investigations and revealed that up-regulation of CD68 on the peripheral blood monocytes is also associated with AD and its psychotic symptoms. Based on the fact that previous studies reported CD68 is an important molecule which induces expression of inflammatory cytokines such as TNF-α and IL-6 in nervous tissues [39–41], it seems that CD68 induces AD and its psychotic symptoms via several mechanisms including induction of inflammation.

In addition, it has been reported that treatment with Risperidone and Quetiapine reduces the production of pro-inflammatory cytokines, such as TNF-α, IL-6 and IFN-γ, and increases secretion of IL-10, as anti-inflammatory cytokine [16,42]. Thus, based on the results of the current study it seems that the anti-psychotic drugs reduce inflammation in the psychotic AD patients in CD68 dependent manner. Although there were no significant relations between use of vitamin B12 and expression of CD68, a reduction of CD68 expression has been detected in the vitamin B12 treated patients. Based on the limited samples in our investigations, it may be concluded that evaluation of the effects of vitamin B12 on the expression of CD68 in a larger sample size may be associated with significant decrease in expression of the molecule.

Data analysis showed that there was a good correlation between percentage of CD68 positive monocytes and intensity of CD68 on the cells. Thus, it seems that both increased and decreased expression of CD68 is associated with increased and decreased number of CD68 positive cells. Results also demonstrated that there were poor to moderate correlations between percentage of CD68 positive monocytes and VAS, MMSE and age and also between intensity of CD68 with CGI in patients treated with the anti-psychotic drugs. Therefore, it seems that CD68 may participate in several complications of psychotic AD patients including dementia, pain and severity of mental disorders symptoms.

The results also showed that there were perfect and moderate negative relations between serum levels of vitamin B12 and CGI in group 4 and BPRS in group 7, respectively. Therefore, it appears that vitamin B12 may be useful to improve severity of mental disorders and also psychiatric symptoms in the AD patients suffering from psychotic symptoms. Moreover, it proves the roles of vitamin B12 in declination of AD symptoms. Based on the fact that serum levels of vitamin B12 have no relation with percentage of CD68 positive monocytes and intensity of CD68, it may be hypothesized that vitamin B12 reduces severity of mental disorders and also psychiatric symptoms in CD68 independent manner.

Finally, based on the results, it may be concluded CD68 is a key molecule which participates in the pathogenesis of aging complications including AD and its complications. Thus, future investigations need to explore the role of CD68 in the immunopathogenesis of AD.

Table 5
Correlations among percentage of CD68 positive monocytes, GMFI of CD68 and serum levels of vitamin B12 with age, VAS, CGI, BPRS and MMSE in group 6.

		Percentage of CD68 positive monocytes	CD68 GMIF	Vitamin B12 serum levels	Age	VAS	CGI	BPRS	MMSE
Percentage of CD68 positive monocytes	Spearman correlation	1	0.085	−0.015	−0.320	−0.211	0.208	0.087	−0.165
	p value		0.755	0.958	0.227	0.433	0.440	0.747	0.541
CD68 GMFI	Spearman correlation	0.085	1	−0.048	−0.159	0.382	0.296	0.323	−0.386
	p value	0.755		0.869	0.556	0.145	0.265	0.222	0.140
Vitamin B12 serum levels	Spearman correlation	−0.015	−0.048	1	0.169	0.331	−0.098	0.055	0.064
	p value	0.958	0.869		0.562	0.248	0.740	0.852	0.827

Spearman's correlation test showed that there were poor correlations between percentage of CD68 positive monocytes and GMFI of CD68 with age, VAS, CGI, BPRS and MMSE in group 6.
GMFI: geometric mean fluorescence intensity, VAS: Visual Analogue Scale, CGI: Clinical Global Impression, BPRS: Brief Psychiatric Rating Scale, MMSE: Mini–Mental State Examination.

Table 6
Correlations among percentage of CD68 positive monocytes, GMFI of CD68 and serum levels of vitamin B12 with age, VAS, CGI, BPRS and MMSE in group 7.

		Percentage of CD68 positive monocytes	CD68 GMIF	Vitamin B12 serum levels	Age	VAS	CGI	BPRS	MMSE
Percentage of CD68 positive monocytes	Spearman correlation	1	0.113	−0.015	−0.019	0.268	−0.303	−0.362	0.621
	p value		0.740	0.958	0.956	0.425	0.366	0.273	0.042
CD68 GMFI	Spearman correlation	0.113	1	−0.048	0.212	0.347	0.560	0.364	−0.466
	p value	0.740		0.869	0.531	0.296	0.073	0.271	0.149
Vitamin B12 serum levels	Spearman correlation	0.013	−0.407	1	−0.040	−0.060	−0.269	−0.560	0.313
	p value	0.964	0.149		0.892	0.840	0.352	0.037	0.276

Spearman's correlation test revealed that there was moderate to good positive correlation between percentage of CD68 positive monocytes and MMSE ($r = 0.621$, $p = 0.042$) and also between concentration of CD68 and CGI in group 7 ($r = 0.560$, $p = 0.073$), while, there was a moderate negative correlation between serum levels of vitamin B12 and BPRS ($r = -0.560$, $p = 0.037$).
GMFI: geometric mean fluorescence intensity, VAS: Visual Analogue Scale, CGI: Clinical Global Impression, BPRS: Brief Psychiatric Rating Scale, MMSE: Mini–Mental State Examination.

Conflict of interest

There is no conflict of interest to declare.

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